0273-2289/04/119/0013/$25.00

# Effect of Detoxification of Dilute-Acid Corn Fiber Hydrolysate on Xylitol Production

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**Received July 2003; Revised April 2004; Accepted April 2004**

## Abstract

Four different detoxification methods were evaluated for the production of xylitol from corn fiber dilute-acid hydrolysate using *Candida tropicalis*. Although *C. tropicalis* could ferment the dilute partially neutralized hydrolysate to xylitol in low yields (0.1 g/g), it could not ferment the concentrated hydrolysate. Overliming, calcium hydroxide neutralization, neutralization combined with activated charcoal, and overliming combined with activated charcoal methods were used to improve the fermentation of the concentrated hydrolysates. The partial neutralization combined with activated charcoal treatment was the most effective method with respect to xylitol yield and productivity. The highest xylitol yield (0.4 g of xylitol/g of xylose) was obtained for the highest concentration of hydrolysate (three times the original) that had been treated with calcium hydroxide and activated charcoal. The corresponding productivity was 0.23 g/(L·h). Overliming caused reduction in xylitol yield.

**Index Entries:** Detoxification; overliming; *Candida tropicalis*; corn fiber; hydrolysate.

## Introduction

Xylitol is a naturally occurring sugar alcohol with unique pharmacologic properties. Its high sweetening power, cariostatic properties, negative heat of solution, and potential application in diabetic food products *(1)* makes xylitol an attractive sucrose substitute in a wide variety of food and oral hygiene products. There are several other potential products that could

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be produced from xylitol if it could be produced at a competitive cost like other polyols.

Hydrolysates of corn fiber, corncob, sugarcane bagasse, and birch wood are potential xylose-rich feedstocks that could be used for either chemical or biochemical production of xylitol. Biologically, yeasts *(2–5)*, filamentous fungi *(6)*, and bacteria *(7–9)* are known to be capable of reducing xylose to xylitol. Production of hydrolysates involves thermochemical, biologic, or mechanical pretreatment of the lignocellulosic feedstock for effective conversion into xylose. Production of hemicellulose hydrolysates by any of the currently known lignocellulosic pretreatment methods *(10–14)* introduces byproducts, which are toxic to fermenting microorganisms *(15–20)*. The microbially toxic compounds derive from either lignocellulosic extractives or degradation products of hemicellulose and lignin *(16)*. The toxic compounds include furans, aromatic aldehydes, aromatic carboxylic acids, aliphatic acids, macromolecules, and other unidentified degradation products *(16,20,21)*. The composition of the toxic products is a function of the pretreatment method and the feedstock. The degree of toxicity of the compounds in the hydrolysates varies for different microorganisms. Thus, to ferment the hemicellulose hydrolysates, the substrate must be detoxified using suitable methods to enable fermentative microorganisms to grow and ferment the substrate. Several detoxification methods have been used including overliming *(17,18,22)*, steaming and evaporation *(17,22)*, solvent extraction *(23)*, and fungal treatment *(17,24)*. The effectiveness of each method depends on the substrate and the microorganism.

Our overall goal is to convert corn fiber hydrolysate into xylitol using fermentation technology. In this article, we report detoxification and fermentation of corn fiber dilute-acid hydrolysate to xylitol using *Candida tropicalis* as the biocatalyst.

## Materials and Methods

### *Corn Fiber*

Wet corn fiber was obtained from Minnesota Corn Processors (Marshall, MN). The corn fiber was air-dried at room temperature to equilibrium moisture content and knife-milled to pass 20-mesh screen. The milled samples were stored in a refrigerator prior to the experiments.

### *Analysis of Corn Fiber*

The corn fiber samples were analyzed for moisture, ash, acid-insoluble material, glucose, xylose, and arabinose contents using American Society for Testing and Materials (ASTM) standard methods. ASTM standard method E 1755-95 *(25)* was used for the determination of ash in the corn fiber. This method covers the determination of ash, expressed as the mass percent of residue remaining after dry oxidation at 575 ± 25°C. ASTM standard method E 1758-95 *(26)* was used to determine the total carbohydrates

present in the corn fiber, and the acid-insoluble residue was determined using ASTM standard method E 1721-95 *(27)*. All the results were reported relative to the 105°C oven-dried mass of the sample. All determinations were carried out in triplicate.

### *Dilute-Acid Hydrolysis of Corn Fiber*

A full factorial experimental design was used for hydrolysis of the corn fiber. For statistical integrity, the plan was repeated two times. The first influential parameter, $H_2SO_4$ concentration, was set at 0.25, 0.5, 0.75, and 1% (v/v). Hydrolysis time of the experiment was set at 15, 30, 45, 60, 75, and 90 min.

The dried corn fiber was slurried in dilute $H_2SO_4$ in concentrations of 0.25, 0.5, 0.75, and 1%. Thus, 7.5 g of corn fiber was placed in 250-mL flasks and slurried with 50 mL of dilute acid. The flasks were covered with aluminum foil and heated in an autoclave at 121°C for 15, 30, 45, 60, or 90 min, depending on the experimental design. The autoclave was operated in the liquid cycle to reduce liquid loss. The flasks were weighed before and after the autoclave cycle to the nearest 0.1 mg. When liquid was lost, the weight was corrected with makeup distilled water. The slurry was centrifuged (3000*g*, 20 min) and vacuum filtered. One part of the filtrate was kept as unconcentrated hydrolysate and designated as H1. The rest of the filtrate was concentrated using a rotary vacuum evaporator at 40°C, 80 rpm, and 84 kPa. One drop of Sigma 289 antifoaming agent per 100 mL of hydrolysate was added before concentration of the hydrolysate. After the concentration, the hydrolysate was centrifuged (3000*g*, 20 min) and vacuum filtered. The filtrate that was concentrated to twice the original concentration was designated H2 and that concentrated to three times the original concentration was designated H3. Table 1 provides the composition of H1, H2, and H3.

### *Detoxification of Hydrolysates*

To reduce the effect of microbial inhibitors caused by the acid hydrolysis, partial neutralization, overliming, and activated charcoal treatments were used. These procedures were used as single treatment or combinations of several methods, as shown in Table 2.

### *Partial Neutralization*

The hydrolysates (H1, H2, H3) were partially neutralized with $Ca(OH)_2$ to pH 4.0. When the pH was stable, the hydrolysates were centrifuged (3000*g*, 20 min) and vacuum filtered. These samples were used for the fermentation studies.

### *Overliming*

$Ca(OH)_2$ was added to the hydrolysates (H1, H2, H3), with stirring, until reaching pH 10.0. Then the hydrolysates were centrifuged (3000*g*, 20 min) and vacuum filtered. Concentrated $H_2SO_4$ was used to adjust the pH to 4.0.

Table 1
Composition of Original and Concentrated Corn Fiber Hydrolysates Treated Under Different Conditions

| Component | $Ca(OH)_2$ | $Ca(OH)_2$ + activated carbon | Overlimed | Overlimed + activated carbon | NaOH[a] |
|---|---|---|---|---|---|
| 1X concentrated hydrolysate (H1) | | | | | |
| Glucose (%) | 9.56 ± 1.78 | 7.31 ± 2.86 | 7.47 ± 0.39 | 6.43 ± 0.59 | na |
| Xylose (%) | 19.86 ± 1.22 | 15.56 ± 2.40 | 18.60 ± 0.59 | 15.61 ± 1.37 | na |
| Arabinose (%) | 15.48 ± 2.14 | 12.00 ± 0.96 | 13.09 ± 1.04 | 11.31 ± 0.25 | na |
| 2X concentrated hydrolysate (H2) | | | | | |
| Glucose (%) | 18.55 ± 2.17 | 17.26 ± 2.51 | 16.13 | 16.24 ± 3.32 | na |
| Xylose (%) | 37.78 ± 0.89 | 33.72 ± 0.84 | 36.63 | 31.50 ± 1.64 | na |
| Arabinose (%) | 26.71 ± 0.36 | 22.55 ± 0.96 | 24.88 | 21.55 ± 2.13 | na |
| 3X concentrated hydrolysate (H3) | | | | | |
| Glucose (%) | 25.93 | 22.24 | 21.29 | 20.02 | 33.60 ± 0.02 |
| Xylose (%) | 56.10 | 48.21 | 43.66 | 40.29 | 60.33 ± 0.64 |
| Arabinose (%) | 38.32 | 31.89 | 34.12 | 29.90 | 41.57 ± 0.12 |

[a]na, no runs.

Table 2
Scheme for Detoxifying Corn Fiber Hydrolysates

| Detoxification method | Substrates treated |
|---|---|
| Neutralized with $Ca(OH)_2$ | H1, H2, H3 |
| Overlimed with $Ca(OH)_2$ | H1, H2, H3 |
| Neutralized with $Ca(OH)_2$ and activated charcoal treatment | H1, H2, H3 |
| Overlimed with $Ca(OH)_2$ and activated charcoal treatment | H1, H2, H3 |
| Neutralized with NaOH and activated charcoal treatment | H3 |

The hydrolysates were centrifuged (3000*g*, 20 min) and vacuum filtered again. These samples were used for the fermentation studies.

### *Activated Charcoal Treatment*

After either the partial neutralization or overliming, 50 g/L of activated charcoal was added to the hydrolysates and stirred for 1 h. The mixture was centrifuged (3000*g*, 20 min) and vacuum filtered. The treated hydrolysate was then used for the fermentation studies. A set of H3 samples was neutralized with NaOH and then treated with the activated charcoal and separated as just described. This filtrate was also fermented. The NaOH treatment was used to assess the effect of differentions on the fermentation of the H3 hydrolysate.

### *Microorganism*

*C. tropicalis* ATCC96745 was acquired from the American Type Culture Collection (Manassas, VA) and used in all the studies. The culture was maintained on yeast extract-peptone-dextrose agar plates at 4°C. Subcultivation was done every 2 wk to maintain viability.

### *Culture Media*

The preculture medium consisted of 60 g/L of xylose, 10 g/L of yeast extract, 15 g/L of $KH_2PO_4$, 3 g/L of $(NH_4)_2HPO_4$, and 1 g/L of $MgSO_4 \cdot 7H_2O$ *(5)*. The pH was adjusted to 5.0 using 1 *M* HCl. The salts and the yeast extract and the xylose were sterilized separately. Concentrated and sterilized solutions were used to adjust the preculture medium.

The production medium contained 15 g/L of $KH_2PO_4$, 3 g/L of $(NH_4)_2HPO_4$, 1 g/L of $MgSO_4 \cdot 7H_2O$, and 20 g/L of yeast extract. The sugar concentrations (arabinose, glucose, and xylose) depended on the concentration of the hydrolysate in each experiment.

### *Fermentation Conditions*

The precultures were grown in 500-mL flasks agitated at 130 rpm on a rotary platform shaker (Innova 2000; New Brunswick Scientific, Edison, NJ) enclosed in a biologic incubator (Model I-60; Percival, Perry, IA) at 30°C. The cells were harvested after 24 h in the exponential growth phase and

separated by centrifuging for 10 min at 3800*g*. The cells were resuspended with sterile water and centrifuged again for 10 min.

The inoculum was transferred to the production medium and adjusted to the desired cell concentration (1 g/L). The medium volume of 40 mL was composed from 32.5 mL of hydrolysate (yeast extract and salts included), 5 mL of inoculum, and about 2.5 mL of HCl or NaOH to adjust the pH to 4.0 *(28)*. Fermentation was carried out in 250-mL cotton-plugged Erlenmeyer flasks at 30°C and agitated at 130 rpm on a rotary platform shaker. Samples were taken every 6 h during the first 24 h of fermentation because the glucose was rapidly depleted. The fermentation of xylose and especially arabinose was slow, so the sampling intervals were increased to 24 and then 72 h when arabinose was the only sugar left in the broth. The sample volume was 300 µL, and samples were kept in a deep freezer (–20°C) until the time of analysis. The experiments were repeated except for samples that had no cell growth.

### *Determination of Dry Cell Mass*

For the spectrophotometric measurement, the samples were diluted with deionized water until they were within the linear range of the spectrometer. After determining the absorbance at 640 nm (Spectronic 1001; Milton Roy, Rochester, NY), the samples were dried in a laboratory gravity oven (Precision Scientific, Chicago, IL) at 80°C to a constant weight. The dry cell mass was determined from the optical density–dry cell calibration curve.

### *Analysis of Samples*

Reducing sugar and sugar alcohols were determined using a Shimadzu VP 10A (Shimadzu, Columbia, MD) high-performance liquid chromatograph equipped with a refractive index detector, an autosampler, and a carbohydrate $Ca^{++}$ column. The column temperature was 80°C, and deionized water was used as the mobile phase at a constant flow rate of 4 mL/min. For the high-performance liquid chromatography analysis, the hydrolysate samples were diluted 1:10 with deionized water and then centrifuged at 3000*g* for 20 min and filtered through a 0.2-µm syringe filter. The injection volume was 10 µL. Shimadzu Class VP software was used for data retrieval and analysis.

The dinitrosalicylic acid method *(29)* was used for determining reducing sugar. A glucose calibration curve was made, and the samples were diluted so that the expected concentration was within the range of the calibration curve. The absorbance was measured at 550 nm.

## Results

### *Composition of Corn Fiber*

The moisture content of the corn fiber was relatively low (3.2%) because of the air-drying and storage in the refrigerator. Table 3 provides

Table 3
Composition of Corn Fiber
on Moisture-Free Basis

| Component | Composition (%) |
|---|---|
| Glucose | 35.32 |
| Xylose | 30.62 |
| Arabinose | 12.84 |
| Acid-insoluble residue | 8.67 |
| Ash | 0.13 |
| Unknown | 12.42 |
| Total | 100.00 |

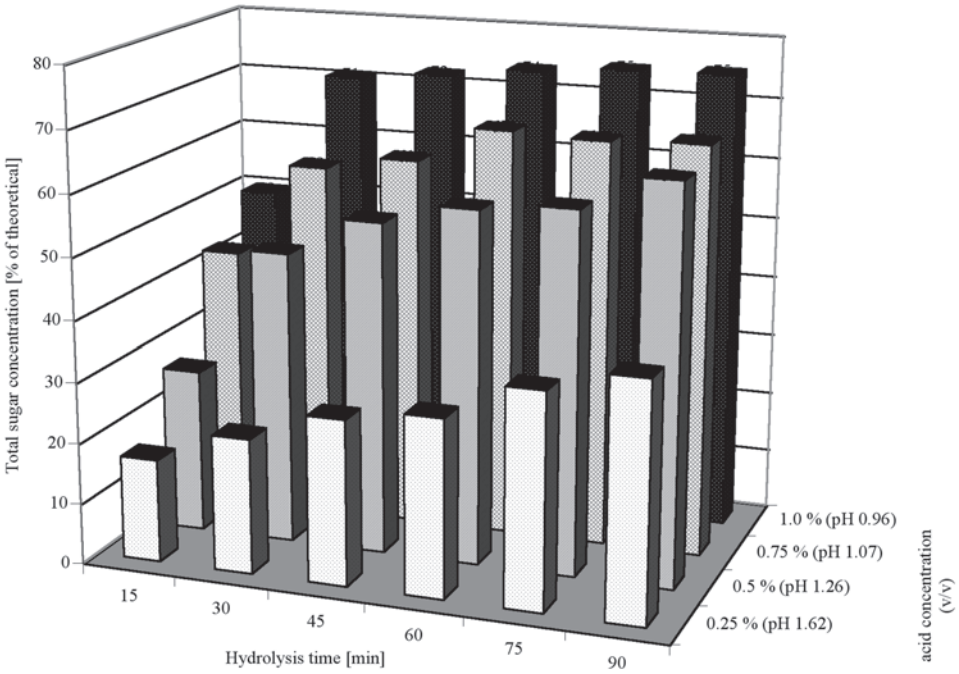


Fig. 1. Effect of $H_2SO_4$ concentration and hydrolysis time on total sugar yield.

the partial summative composition of the corn fiber. The high glucose content was attributed to the residual starch in the corn fiber. However, the starch content of the feedstock was not determined.

## *Dilute-Acid Hydrolysis of Corn Fiber*

The effects of hydrolysis time (15–90 min) and dilute $H_2SO_4$ concentration (0.25–1%) on total sugar yields are shown in Fig. 1. The yields are expressed as a percentage of the maximum theoretical yield. The total monomeric sugar yield was a function of both the hydrolysis time and the

Table 4
Regression Equations for Dilute-Acid Hydrolysis Products of Corn Fiber

| Parameter | Regression equation[a] | $R^2$ |
|---|---|---|
| Xylose (g/L) | $-25.68 + 100.40C_a + 0.52t - 53.52C_a^2 - 0.0025t^2 - 0.18t \cdot C_a$ | 0.95 |
| Glucose (g/L) | $-22.08 + 32.78C_a + 0.59t - 0.0037t^2$ | 0.94 |
| Arabinose (g/L) | $12.04 + 10.28C_a + 0.03t - 4.80C_a^2 - 0.18C_a^2 \cdot t$ | 0.79 |
| Furfural (g/L) | $-0.15 + 0.175t \cdot C_a + 0.00002t^2$ | 0.99 |
| HMF (g/L) | $-0.054 + 0.141C_a - 0.101C_a^2 + 0.0043C_a \cdot t$ | 0.98 |

[a]In the equations, $C_a$ is the initial concentration of $H_2SO_4$ (% v/v), and $t$ is the hydrolysis time (min).

acid concentration. At low acid concentration (0.25%), increasing the hydrolysis time from 15 to 90 min increased the total sugar yield from 17 to 39%, whereas at high acid concentration (1.0%), increasing the hydrolysis time from 15 to 90 min increased the sugar yield from 50 to 75%. On the other hand, increasing the acid concentration from 0.25 to 1% for a short hydrolysis time (15 min) increased the sugar yield threefold, whereas for a 90-min hydrolysis time the same change in acid concentration (0.25–1.0%) increased the sugar yield 1.9-fold. The difference in yields could be due to the degradation of the sugars to furfural and hydroxymethylfurfural (HMF) at longer hydrolysis times.

Polynomial models were used to establish the relationship between the monomeric hydrolysis products and the independent variables (hydrolysis time and acid concentration). The data were fitted to Eq. 1:

$$C_i = \beta_0 + \beta_1(C_a) + \beta_2(t) + \beta_3(C_a \times t) + \beta_4(C_a^2) + \beta_5(t^2) \quad (1)$$

in which $C_i$ is the concentration (g/L) of xylose, glucose, arabinose, furfural, or HMF; $C_a$ is the concentration of $H_2SO_4$ (% v/v); $t$ is the hydrolysis time (min); and $\beta_0$, $\beta_1$, $\beta_2$ … are constants.

The xylose concentration of the hydrolysate was fitted to a second-order polynomial model (Table 4). Analysis of variance showed that the linear and quadratic terms were significant ($p < 0.05$). The xylose concentration was a function of both acid concentration and hydrolysis time. However, the concentration of the acid had a stronger influence on the yield of xylose than hydrolysis time. The highest xylose yield was 73%.

The yield of glucose was also modeled by a second-order polynomial (Table 4) and was influenced by both the hydrolysis time and the concentration of the acid. Similar to the xylose, the influence of hydrolysis time was most effective at low acid concentrations. Acid concentration appeared to have a stronger influence on glucose production than hydrolysis time. Unlike xylose production, the interaction parameter was not statistically significant. The highest glucose yield was 72% of the theoretical yield.

The yield of arabinose was not strongly affected by either the hydrolysis time or the acid concentration. The second-order polynomial model showed a weak $R^2$ statistic (Table 4). The yield ranged between 71 and 90%.

The highest yield of arabinose was obtained at 0.5% $H_2SO_4$ and 30-min hydrolysis time, and the lowest yield was obtained at 0.25% $H_2SO_4$ and 15-min hydrolysis.

The concentrations of furfural and HMF in the hydrolysates were also measured. The production of these compounds was also hydrolysis time and acid concentration dependent but was more influenced by the interaction parameters (Table 4). This implies that the acid and hydrolysis time parameters can be manipulated to minimize the production of these degradation products. Both furfural and HMF production were highest at the highest acid concentration and the longest hydrolysis time.

### *Partial Neutralization of Corn Fiber Hydrolysates*

The effect of partial neutralization with $Ca(OH)_2$ on the toxicity of the hydrolysate to *C. tropicalis* growth and fermentation was a function of the concentration of the hydrolysate. For the unconcentrated hydrolysate, H1 (Table 1), it appeared that the inhibitor concentration was so low that the partial neutralization with $Ca(OH)_2$ was sufficient for effective cell growth and nutrient uptake. The lag phase for this substrate was 16 h (Table 5). Unlike model sugar studies that showed diauxic growth *(28)*, there was simultaneous uptake of all sugars. Although the xylose uptake rate was very high and there appeared to be no catabolite repression from either the glucose or the other sugars present, the xylitol yield was very low. Greater than 90% of the xylose was consumed within 50 h of fermentation, yielding only 0.1 g of xylitol/g of xylose.

Similar to model sugar studies, glucose utilization was very rapid and produced ethanol. Most glucose was used within 24 h, producing about 5 g/L of ethanol. However, when all the glucose was utilized, ethanol production continued at a reduced rate and reached a maximum of 7 g/L. The rate of arabinose utilization was very slow, and arabitol yield was relatively high although the productivity was lower than that of xylitol.

When the partially neutralized concentrated hydrolysate (H2) was fermented, the lag period was 84.7 h compared with 16 h for H1. The specific growth rate and xylitol productivity were similar to those for H1, but xylitol yield (0.17 g/g) was higher (Table 5).

When the hydrolysate concentration was increased to three times the original hydrolysate (H3), *C. tropicalis* could neither grow nor produce xylitol in the partially neutralized hydrolysate. Clearly, it appeared that only some fraction of the inhibitors was removed by the partial neutralization. The inhibitor concentration appeared to be higher than the threshold level for cell growth.

### *Activated Charcoal Treatment of Partially Neutralized Hydrolysates*

Activated charcoal treatment of the partially neutralized samples produced very interesting results. For the H1 samples, the lag period of 16 h was reduced to zero and specific growth rate increased from 0.062 to 0.094/h. Xylitol yield doubled, and productivity increased more than twofold rela-

Table 5
Yields and Productivities After Inhibitor-Removing Treatment

| Concentration factor | Pretreatment | Lag phase (h) | Specific growth rate ($h^{-1}$) | Ethanol[a] productivity[2)] ($g_{Ethanol}/[L{\cdot}h]$) | Xylitol | | Arabitol | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Yield[2)] ($g_{Xylitol}/g_{Xylose}$) | Productivity[2)] ($g_{xylitol}/[L{\cdot}h]$) | Yield[2)] ($g_{Arabitol}/g_{Arabinose}$) | Productivity[2)] ($g_{Arabitol}/[L{\cdot}h]$) |
| H1 | Partially neutralized with $Ca(OH)_2$ | 16.00 | 0.06 | 0.15 | 0.09 | 0.03 | 0.38 | 0.03 |
| H2 | Partially neutralized with $Ca(OH)_2$ | 84.75 | 0.06 | 0.11 | 0.17 | 0.04 | 0.29 | 0.01 |
| H3 | Partially neutralized with $Ca(OH)_2$ | — | 0 | 0 | 0 | 0 | 0 | 0 |
| H1 | Partially neutralized with $Ca(OH)_2$ + activated charcoal treatment | 0 | 0.09 | 0.17 | 0.17 | 0.08 | 0.74 | 0.10 |
| H2 | Partially neutralized with $Ca(OH)_2$ + activated charcoal treatment | 0 | 0.10 | 0.23 | 0.22 | 0.13 | 0.86 | 0.18 |
| H3 | Partially neutralized with $Ca(OH)_2$ + activated charcoal treatment | 16.00 | 0.07 | 0.22 | 0.40 | 0.23 | 0.76 | 0.08 |
| H1 | Overlimed with $Ca(OH)_2$ | 41.25 | 0.07 | 0.10 | 0.11 | 0.03 | 0.52 | 0.03 |
| H2 | Overlimed with $Ca(OH)_2$ | — | 0 | 0 | 0 | 0 | 0 | 0 |
| H3 | Overlimed with $Ca(OH)_2$ | — | 0 | 0 | 0 | 0 | 0 | 0 |
| H1 | Overlimed with $Ca(OH)_2$ + activated charcoal treatment | 0 | 0.09 | 0.20 | 0.12 | 0.05 | 0.72 | 0.09 |
| H2 | Overlimed with $Ca(OH)_2$ + activated charcoal treatment | 9.50 | 0.10 | 0.26 | 0.21 | 0.12 | 0.86 | 0.18 |
| H3 | Overlimed with $Ca(OH)_2$ + activated charcoal treatment | 47.10 | 0.10 | 0.21 | 0.31 | 0.20 | 0.64 | 0.08 |
| H3 | Partially neutralized with NaOH + activated charcoal treatment | 67.50 | 0.04 | 0.13 | 0.40 | 0.130 | 0.56 | 0.03 |

[a]Ethanol yield was not calculated, because it was produced from two substrates (glucose and xylose). [2]Yield and productivities are calculated at the highest product concentration.

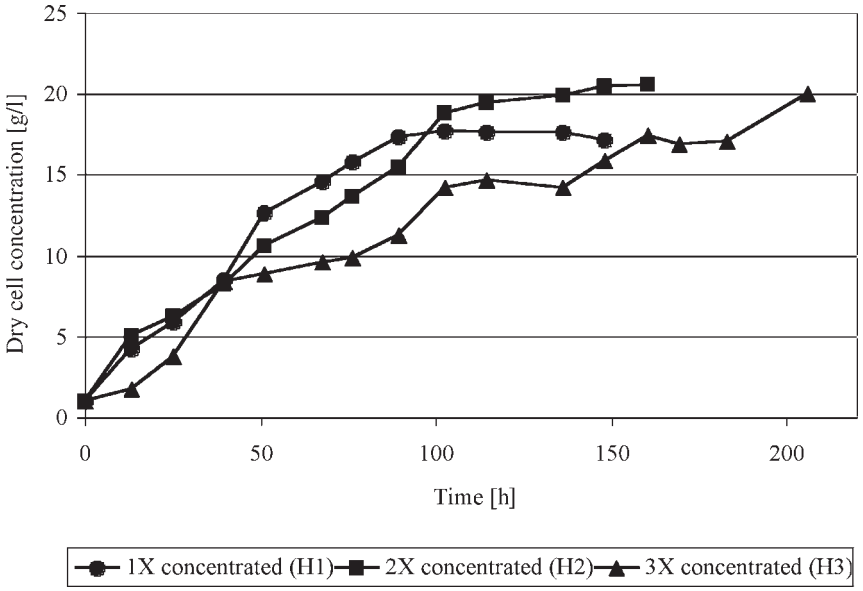


Fig. 2. Cell growth of *C. tropicalis* on $Ca(OH)_2$ partially neutralized and activated charcoal-treated corn fiber hydrolysate.

tive to the partially neutralized samples that were not treated with the activated charcoal (Table 5). The sugar uptake was simultaneous and glucose was completely utilized within the first 20 h. The initial product was mostly ethanol (5 g/L), with a small amount of xylitol (0.8 g/L). After depletion of the glucose, ethanol production continued slowly until it reached a maximum of 7 g/L in 44 h. Appreciable accumulation of xylitol occurred only after the exhaustion of the glucose. When xylose and other sugars in the medium were exhausted after 50 h of fermentation, ethanol and xylitol were utilized for cell growth. Thus, cell mass accumulation continued until these substrates were also exhausted (Fig. 2).

The activated charcoal treatment of the H2 samples reduced the lag phase of 84.75 h for the partially neutralized sample to zero for the treated sample. The specific growth rate; yields; and productivities of xylitol, arabitol, and ethanol all increased considerably with treatment (Table 5). Similar to the H1 samples, although there was simultaneous uptake of the sugars, the initial product formation within the first 20 h was ethanol, which derived from glucose metabolism. All the glucose was depleted within 20 h of fermentation, and the ethanol concentration was 8 g/L and xylitol concentration was 0.3 g/L. Ethanol production continued after the exhaustion of the glucose until a maximum concentration of 13 g/L was attained after 60 h of fermentation. Appreciable xylitol accumulation only started after the exhaustion of the glucose. The maximum xylitol accumulated was 6 g/L, which was double that obtained for the H1 sample. However, both xylitol and ethanol yields declined after the exhaustion of all the sugars in the medium because *C. tropicalis* started to use these products for cell

growth. Cell mass accumulation therefore continued after exhaustion of the hydrolysate sugars (Fig. 2).

In the case of H3 samples, which could not be fermented when they were only partially neutralized, treatment with activated charcoal resulted in cell growth and fermentation of xylose to xylitol. However, there was a lag period of 16 h, which implied that there were some residual inhibitors in the hydrolysate. Apart from the lag phase, the trends in sugar uptake; sugar utilization; and production of xylitol, ethanol, and arabitol were similar to those observed for the H1 and H2 samples. Cell mass accumulation was similar to H1 and H2 (Fig. 2). The specific growth rate of cells in the H3 samples was slightly less than for the H1 and H2 samples. The xylitol yield and productivity were more than twice those obtained for the H1 samples (Table 5). The highest ethanol concentration in the fermentation broth was 18 g/L, but ethanol productivity was similar to that of the H2 samples. The arabitol yield and productivity were lower than those for the H2 samples.

The H3 samples that were partially neutralized with NaOH coupled with treatment with activated charcoal had a lag period of 67.5 h compared with 16 h for the corresponding $Ca(OH)_2$-treated samples (Fig. 3). The specific growth rate was reduced to one-half the corresponding sample partially neutralized with $Ca(OH)_2$. Xylitol yield was similar (0.4 g/g), but xylitol productivity was reduced considerably. Similar to the $Ca(OH)_2$ partially neutralized samples, ethanol was initially produced until all the glucose was exhausted before appreciable xylitol accumulation began. Arabinose was not utilized until all the xylose, glucose, and other sugars were exhausted. The rate of arabinose utilization was very slow and occurred simultaneously with the utilization of ethanol. Under these conditions, xylitol utilization was minimal compared to the $Ca(OH)_2$-treated samples. Further, ethanol productivity as well as arabitol yield and productivity were reduced considerably.

## *Overliming of Corn Fiber Hydrolysate*

Similar to the $Ca(OH)_2$ partial neutralization treatment, the overlimed H1 hydrolysate could support cell growth and xylitol promotion. However, the lag phase (41.25 h) was longer than that for the partially neutralized sample (Table 5). Although there was simultaneous sugar uptake, no appreciable xylitol was accumulated until all the glucose was exhausted. The initial product was ethanol from the glucose metabolism and xylitol accumulation was very low (0.5 g/L). The specific growth rate and xylitol yield were slightly higher than those for the partially neutralized samples. The xylitol yield and productivity were 0.11 g of xylitol/g of xylose, and 0.03 g/(L·h), respectively.

The overlimed H2 and H3 samples could neither support cell growth nor produce xylitol. This was rather surprising because overliming has been reported as an effective procedure for removing microbial growth and fermentation inhibitors *(16–18,20)*.

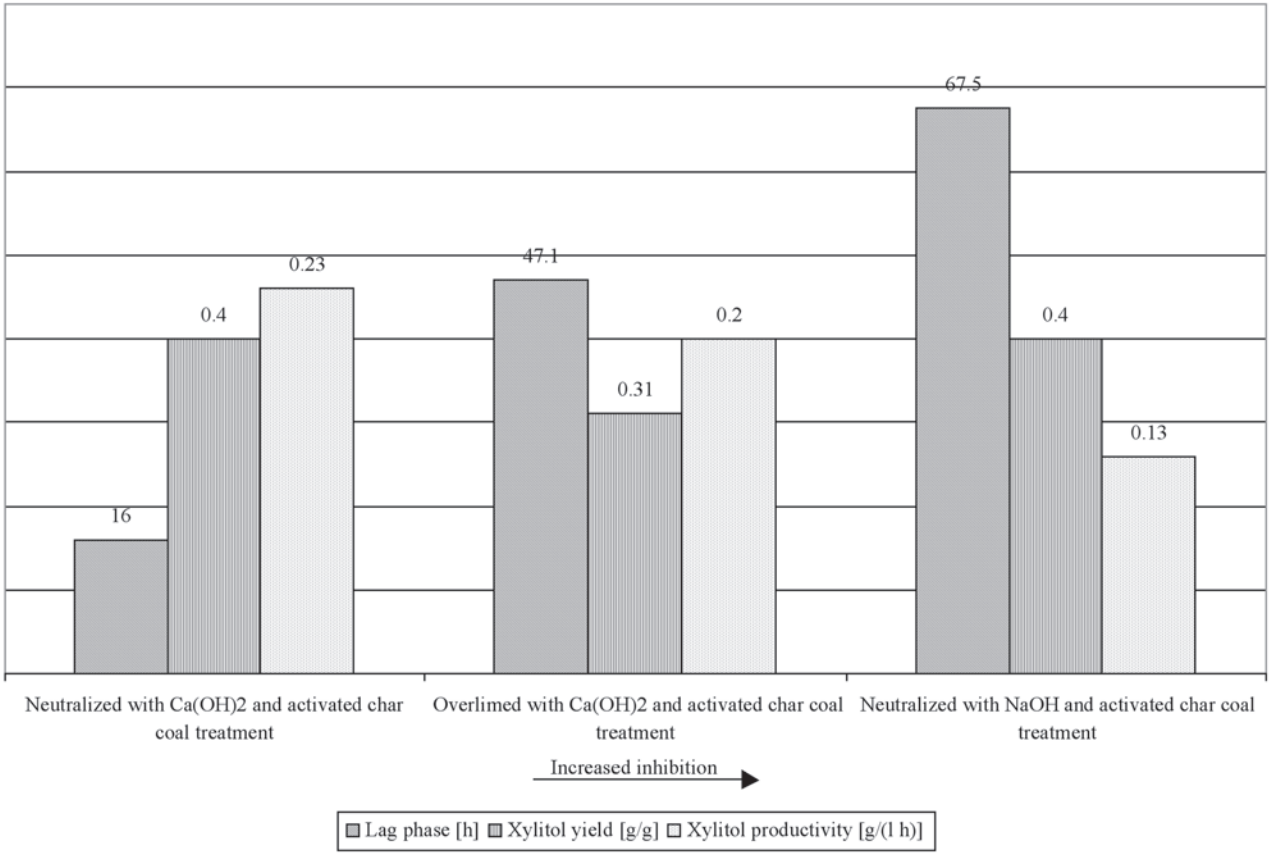


Fig. 3. Effect of detoxification method of H3 samples on lag phase, xylitol yield, and productivity.

### *Activated Charcoal Treatment of Overlimed Hydrolysates*

The activated charcoal treatment of overlimed H1, H2, and H3 improved the performance of *C. tropicalis* although this was less effective compared to the partially neutralized samples. The lag period of 41.25 h observed for overlimed H1 samples was reduced to zero after activated charcoal treatment. Although there was a simultaneous uptake of the sugars, ethanol (4.5 g/L) was the major product accumulated within the first 19 h of fermentation whereas xylitol (0.88 g/L) was low. Glucose was completely utilized within the first 19 h, but ethanol production continued until the concentration rose to 5.8 g/L after 44 h of fermentation. Xylitol accumulation did not rise above 1 g/L and started to decrease after 44 h fermentation. Both xylitol and ethanol were used for cell growth after the sugars were depleted. Arabinose utilization was only appreciable after the depletion of the other sugars. Arabinose was used simultaneously with ethanol and xylitol. Specific growth rate, arabitol yield, and ethanol and arabitol productivities increased relative to the overlimed samples after activated charcoal treatment (Table 5). Xylitol yield and productivity did not change compared to the samples not treated with activated charcoal.

The overlimed H2 and H3 samples that could not support cell growth and fermentation could support cell growth and xylitol production after activated charcoal treatment. However, for both H2 and H3 there were lag phases of 9.5 and 47.1 h, respectively. Trends in sugar utilization; cell growth; and xylitol, ethanol, and arabitol production were similar to those of the $Ca(OH)_2$ partially neutralized and activated charcoal–treated samples.

## Discussion

The toxicity of biomass hydrolysates has been reported to be both biomass and microorganism dependent *(16)*. For most ethanologens, overliming with calcium salts has been reported to be highly effective for detoxifying biomass hydrolysates for fermentation to ethanol. Overliming was also found to be effective in the production of xylitol from bagasse hydrolysate using *Candida guilliermondii (30)*. However, our current studies show that overliming may not be effective for some microorganisms such as *C. tropicalis*.

The overlimed H2 and H3 samples could neither support cell growth nor produce xylitol, and overlimed H1 samples had a long lag period. Interestingly, when the H2 hydrolysate was partially neutralized with $Ca(OH)_2$, the cells could grow and produce xylitol, and in the case of H1, there was no lag phase. These observations suggest that calcium ions play several roles in the treatment of corn fiber hydrolysates: they neutralize the hydrolysate, form complexes with some inhibitors, and also appear to influence the growth and productivity of *C. tropicalis*.

In the case of the H1 samples, if it is assumed that the concentration of the inhibition compounds was relatively low and that $Ca(OH)_2$ neutralizes

and forms complexes with the toxic compounds, then both the overlimed and partially neutralized H1 samples should have similar fermentation characteristics. The long lag phase for the overlimed sample compared with the neutralized samples clearly showed that excess calcium ions influenced the growth of *C. tropicalis*.

For the overlimed H2 samples, a similar argument could be advanced to support the total inhibition of growth and fermentation. In this case, the total inhibition could be a synergistic effect of the residual toxic compounds of the hydrolysate and the excess calcium ions. This argument is further supported by the activated charcoal treatment of the partially neutralized and overlimed samples. The partially neutralized and activated charcoal-treated samples of H1 and H2 appeared to have either low or no toxic compounds present and, therefore, cells grew very rapidly with no lag phase. For the corresponding overlimed and activated charcoal-treated samples, H1 samples did not show any lag phase, but H2 had a lag phase of 9.5 h for the same inoculum concentration. The H3 samples for both overlimed and partially neutralized samples treated with activated charcoal had lag phases, but the lag phase of the overlimed sample was almost threefold the lag phase of the neutralized sample (Fig. 3). Thus, it appears that although the inhibition from biomass degradation products was reduced by $Ca(OH)_2$, the excess calcium ions were probably toxic to *C. tropicalis*.

These observations suggest that there are two classes of inhibitors in the corn fiber hydrolysates: those that are removed by simple neutralization with $Ca(OH)_2$ and those that can only be removed by activated charcoal. $Ca(OH)_2$ appeared to neutralize some toxic components and form complexes with others. This was borne out by the difference in the performance of hydrolysates treated with NaOH/activated charcoal instead of $Ca(OH)_2$/activated charcoal. Because sodium ions are not known to form complexes, the complex-forming inhibitors were still present in the hydrolysate, and this caused an increase in the lag period and reduced the performance of *C. tropicalis*. These complex-forming inhibitors did not appear to interfere with cell growth because the specific growth rate did not change, but all product formation pathways appeared to be affected.

The class of inhibitors removed by the activated charcoal was not affected by overliming. It appeared that the partial neutralization removed as much toxic compound as the overliming process. The excess calcium ions introduced by overliming did not interact with this class of compounds but, rather, interfered with the growth and metabolism of *C. tropicalis*.

Although the combination of activated charcoal and $Ca(OH)_2$ neutralization appears to be effective, it is most effective at low hydrolysate concentrations; at higher concentrations, only some fraction of the toxic compounds was effectively removed by this treatment, which resulted in a lag phase in which the cells adopted to the less hospitable environment.

The low xylitol yield from corn fiber hydrolysate compared to model sugars could be due to several factors, including a low initial xylose concentration, inhibition from ethanol, an increase in the activity of xylitol

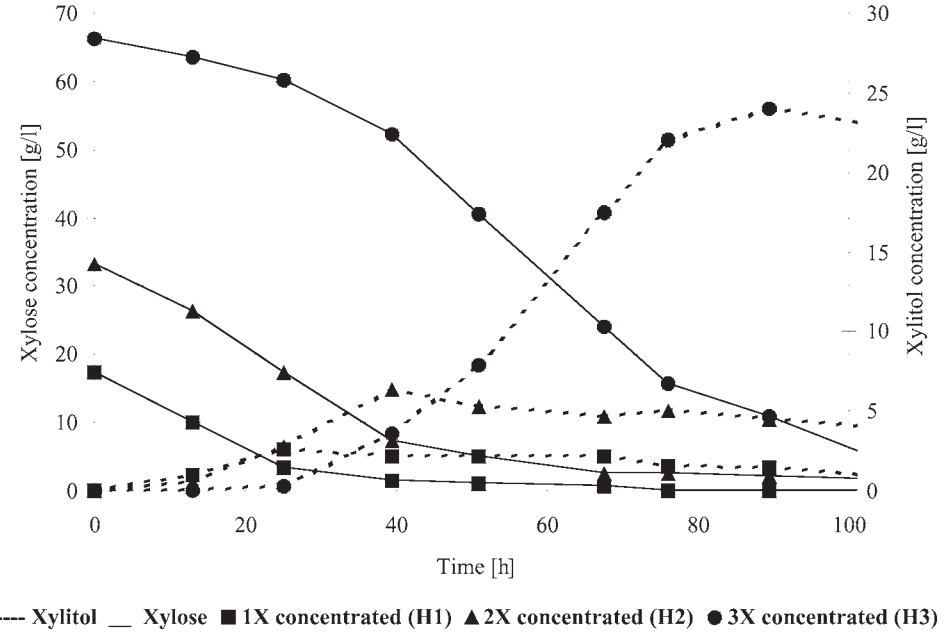


Fig. 4. Xylose utilization and xylitol production from $Ca(OH)_2$ partially neutralized and activated charcoal-treated corn fiber hydrolysate samples.

dehydrogenase in the presence of ethanol, an increase in xylitol dehydrogenase activity because of some biomass degradation products, or a change in the metabolic pathway of *C. tropicalis* owing to compounds in the hydrolysate.

The yield of xylitol from detoxified corn fiber hydrolysate was a function of the initial concentration of xylose in the hydrolysate (Fig. 4). For low xylose concentration (20 g/L of xylose), xylitol yield was very low, but as the initial concentration of xylose increased, the yield of xylitol increased. At an initial xylose concentration of 60 g/L, the yield of xylitol was highest (0.4 g/g), but it was lower than the 0.51 g/g attained with model sugars with similar glucose concentration *(28)*.

Xylitol yields are also influenced by glucose, mannose, and galactose concentrations and the aeration. Glucose and mannose are usually converted into ethanol by *C. tropicalis*, which tends to inhibit xylitol production *(28)*. In our study, ethanol concentration was as high as 18 g/L for the H3 samples. At such high ethanol concentrations, it was shown that xylitol production could decrease by 45% or more depending on the aeration regime *(28)*. Ethanol inhibition is most pronounced under aerobic conditions and least under microaerobic conditions. The volume of the medium used in the current experiments could be classified as aerobic according to the criteria used by Walther et al. *(28)*. Thus, maximum inhibition of xylitol production by ethanol was possible. Further, the presence of other inhibitory compounds in the hydrolysate could have contributed to the low xylitol yield.

## Conclusion

Greater than 73% of the xylose in corn fiber can be recovered in the hydrolysate after dilute $H_2SO_4$ treatment of the substrate. Investigations of the fermentation behavior of *C. tropicalis* showed that the overliming procedure was not effective in removing some class of inhibitors from the acid pretreatment of corn fiber hydrolysate. Consequently, the substrate was still toxic. Additionally, the overliming appeared to induce calcium toxicity in the microorganism. A combination of partial neutralization and activated charcoal treatment was a more effective detoxification method for xylitol production by *C. tropicalis*. Xylitol production strongly depended on the initial xylose concentration, and product formation was inhibited by the ethanol produced from the glucose and mannose in the corn fiber. Thus, when using corn fiber as a raw material for xylitol production, the detoxification procedure will be crucial for its success.

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